The Structure of a Mixed Amino-acid Complex: L-Histidinato-L-threoninatoaquocopper(II) Hydrate

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THE identification of Cu^{II}–amino-acid complexes in normal human serum[1] suggested that these molecules may play a part in the transport of Cu^{II} through the blood and between blood and tissues.[2] Later it was shown (t.l.c.) that a significant proportion of the complexed Cu^{II} was in the form of a mixed-ligand complex containing L-histidine, L-threonine, and Cu^{II} in the ratio 1 : 1 : 1. The equilibria of this complex in solution were studied by spectroscopy[3] and potentiometric titration.[4] We have performed an *X*-ray crystallographic analysis of the complex prepared from a solution of approximately physiological pH.[1]

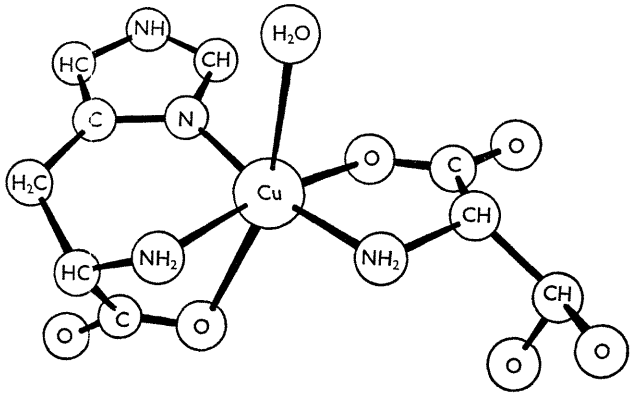

FIGURE. *Structure of* L-*histidinato*-L-*threoninatoaquocopper*(II) *complex.*

The Cu atom has an elongated and distorted octahedral co-ordination geometry. The histidine residue functions as a tridentate chelate. Its N(imidazole) and N(amino) atoms lie at corners of the co-ordination square, 1·95 and 2·01 Å from the Cu atom, respectively. An O(carboxy) atom lies 2·58 Å from the metal in an irregular axial position [angle N(amino)–Cu–O(carboxyl) = 68·3°]. Distorted configurations about Cu^{II} have been observed for other amino-acid[5] and peptide[6] complexes.

The threonine residue, like many other α-amino-acid ligands,[7] binds the Cu through its N(amino) and O(carboxy) atoms to form a five-membered chelate ring (bond-length Cu–N = 2·00, Cu–O = 1·97 Å, angle N–Cu–O = 82·6°). The hydroxy-group is uninvolved, in agreement with the suggestion of Sharma.[8]

So far as the metal–histidine interaction is concerned, the structure of the mixed complex differs from that proposed earlier from the properties of the complex in solution.[3] We have no proof that the species found in the crystalline state is identical with any of the several species which may be present in solution, but it is likely that the co-ordination of histidine does not change at the moment of crystallisation. The structure originally deduced for Cu(L-His)(L-Thr) from solution data owed much to the structure already suggested for $Cu(L\text{-}His)_2$.[9] The discovery that histidine forms two strong Cu–N bonds and an irregular Cu–O bond in crystals of the mixed complex therefore raises the question to what extent the same (hitherto unexpected) co-ordination behaviour may occur in other systems containing Cu^{II} and histidine.

It is clear that, as the pH of a solution is raised, species† such as $Cu(HisH)_2^{2+}$, $Cu(HisH)(His)^+$, and $Cu(HisH)(Thr)^+$ are replaced by such as $Cu(His)_2$ and Cu(His)(Thr), and ultimately by $Cu(His)(ThrH_{-1})^-$, *etc.*[4] Undoubtedly the histidine imidazole group is fully protonated and consequently free at *low* pH.

This is substantiated by the crystal-structure analysis of a complex prepared at pH 3·7, $[Cu(L\text{-}HisH)_2(OH_2)_2](NO_3)_2$,[10] in which the metal is attached only to the N(amino) and O(carboxy) atoms of the amino-acid. The persistence of the Cu–N(amino) bond as the pH is raised has never been questioned, but the continued participation of the histidine carboxy-group in Cu-binding at pH 7·4 has only recently been proved.[9] Earlier workers[11] suggested chelation *via* the amino- and imidazole groups and non-involvement of the carboxy-group. The new evidence for Cu–O(carboxy) bonding consists of a significant shift in the antisymmetric i.r. CO_2^- band in Cu^{II}–histidine solutions.[9] This spectral shift is, however, just as consistent with a Cu–O(carboxy) bond in an irregular axial orientation as with one directed to a corner of the co-ordination square of the Cu atom.

The formation of a strong Cu–N(imidazole) bond and the continued interaction of the Cu atom with the histidine carboxy-group are therefore not mutually exclusive—an assumption implicit in the interpretation of the discontinuous potentiometric and spectrophotometric titrations of the Cu–histidine system[9] and of the o.r.d. curves and visible and e.s.r. spectra of the mixed Cu–histidine–threonine system.[3] More recent measurements[12] of proton displacement as a function of pH have shown that Cu-binding at histidine imidazole groups *does* occur, though the data again cannot discriminate between two alternatives; the new solution data for $Cu(L\text{-}His)_2$ are adequately explained either (i) if one of the histidine ligands is tridentate and the other bidentate, or (ii) if both are tridentate. Tridentate chelation has also been deduced from the o.r.d. and c.d. spectra of Cu^{II}–histidine solutions.[13] The present structure analysis shows how tridentate chelation of Cu^{II} by histidine may be accommodated sterically.‡ Indeed, the pH-dependent displacement of an O(carboxy) donor atom from

† $(HisH) = NH_2{\cdot}CH(CH_2{\cdot}C_3N_2H_4^+){\cdot}CO_2^-$, $(His) = NH_2{\cdot}CH(CH_2{\cdot}C_3N_2H_3){\cdot}CO_2^-$,
$(ThrH_{-1}) = NH_2{\cdot}CH(CHMe{\cdot}O^-){\cdot}CO_2^-$ *or* Thr + OH^-
Thr = $NH_2{\cdot}CH(CHMe{\cdot}OH{\cdot}CO_2^-$, *etc.*

‡ If $Cu(L\text{-}His)_2$ is bis-tridentate then steric considerations show that the arrangement of the four nearest donor atoms must be *cis*.

the co-ordination square of Cu^{II} by N(imidazole) is just what would be expected from the resultant increase in the crystal-field stabilisation energy of Cu^{II}. Continued metal-binding by the O(carboxy) atom, albeit from a sterically unfavourable position, then leads to tridentate chelation which is thermodynamically advantageous ('the chelate effect').

The deep-blue crystals were monoclinic with $a = 5{\cdot}843(10)$, $b = 12{\cdot}249(10)$, $c = 11{\cdot}049(10)$ Å, β 102°46′, D_m 1·60, D_c 1·58 g.cm.$^{-3}$, $Z = 2$ for M 371·5, $\mu = 24{\cdot}2$ cm.$^{-1}$; space-group $P2_1$. 1421 Reflections (69 unobervably weak) were recorded with Cu-K_α radiation on an automated Supper equi-inclination diffractometer. The structure was solved by three-dimensional Patterson and Fourier methods and was refined by full-matrix least-squares to $R = 0{\cdot}038$. The hydrogens (located in a difference synthesis) were included in the final structure-factor calculation.

This work was supported by grants from the Institute of General Medical Sciences, U.S. Public Health Service, and from the Australian Research Grants Committee.

(*Received, December 30th,* 1968; *Com.* 1796.)

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